Preparation and Properties of Chloroplasts Depleted of Chloroplast Coupling Factor 1 by Sodium Bromide Treatment

Received for publication May 21, 1974 and in revised form September 18, 1974

AYALA KAMIEJNETZKY AND NATHAN NELSON
Department of Biology, Technicon-Israel Institute of Technology, Haifa, Israel

ABSTRACT

Chloroplasts were treated with 2 M sodium bromide. The resulting particles lost their ATPase activity and chloroplast coupling factor 1 subunits were detected in the supernatant by means of gel electrophoresis and specific antibodies. The chloroplast coupling factor 1 depleted particles show high rates of Hill reaction with pH optimum shifted toward lower pH. The sodium bromide treatment also abolished the light-induced proton uptake. In the presence of N-methylphenazonium methosulfate light-induced proton release, insensitive to uncouplers, was observed. Addition of dicyclohexylcarbodiimide reversed the light-induced pH changes to the normal proton uptake and increased the pH optimum of the Hill reaction.

The resolution of the energy-transducing system in chloroplasts met more difficulties than in mitochondria. In the latter, particles fully depleted of ATPase (F1) and active in electron transport are available (19). Chloroplast particles depleted of CF1 were prepared by EDTA or silicotungstate treatment (10, 11). The EDTA-treated particles can be reconstituted by addition of CF1; however, the degree of the reconstitution depends on the amount of CF1 retained on the particles (H. Nelson and N. Nelson, unpublished observations; 10, 20). The electron transport properties of these particles were not damaged and they responded like uncoupled chloroplast (2, 8, 11). In silicotungstate-treated particles which are highly depleted of CF1, the electron transport is badly damaged (10).

The need for highly resolved chloroplast particles (16) prompted us to look for a treatment which will deplete all of the CF1 while the electron transport remains intact. It is the purpose of this communication to describe the preparation of NaBr-treated chloroplasts which are fully depleted of CF1 while their electron transport remains intact.

MATERIALS AND METHODS

Tricine, digitonin, ATP, ADP, and BSA were obtained from Sigma. Acrylamide, methylenebisacrylamide, and SDS were obtained from Bio-Rad. Tricine, Tricine-maleate, and Tricine-MES buffers were prepared by adjusting the pH with NaOH.

Phosphorylation (1), O2 evolution (10), proton uptake (17), and ATPase activity (15) were performed by published procedures. [γ-32P]ATP was prepared (15) and NADP photoreduction was measured as previously described (13). Gel electrophoresis in the presence of SDS was performed as described by Weber and Osborn (22). The gels were fixed, stained, and destained as previously described (14).

Preparation of Chloroplasts. About 60 g of lettuce (Lactuca sativa var. romaine) leaves were homogenized in a Waring Blender at low speed for 5 to 8 sec in 200 ml of medium containing 0.4 M sucrose, 10 mM NaCl, 10 mM Tricine (pH 8), 20 mM sodium ascorbate, and 0.5 mg/ml of BSA (Sigma fraction V). The homogenate was filtered through gauze and centrifuged, with SS-34 rotor in RC2-B Sorval centrifuge, until it reached 3000 rpm. The precipitate was discarded, and the supernatant was centrifuged at 1500g for 7 min. The pellet was suspended in 10 mM Tricine (pH 8) and centrifuged at 20,000g for 5 min. The pellet was dispersed by glass-Teflon homogenizer in 5 ml of medium containing 0.4 M sucrose, 10 mM NaCl, 10 mM Tricine (pH 8), and 10 mg/ml of BSA.

Preparation of Sodium Bromide-treated Chloroplasts. Chloroplasts suspended in the above medium but without BSA were incubated with 2 M NaBr at 0 C for 30 min. The NaBr was added as 5 M solution. An equal volume of H2O was added, and the suspension was centrifuged at 35,000g for 15 min. The pellet was suspended in medium containing 0.4 M sucrose, 0.01 M NaCl, 0.01 M Tricine, (pH 8), and 10 mg/ml of BSA to give a Chl concentration of about 1 mg/ml.

RESULTS

Chloroplasts treated with NaBr lost photophosphorylation and heat-activated Ca2+-ATPase activity (Fig. 1). Photophosphorylation was more sensitive than the ATPase to NaBr treatment. At 0.4 M NaBr about 60% of the Ca2+-ATPase activity was retained while the photophosphorylation was almost fully inhibited. However, treatment with 2 M NaBr completely inhibited both reactions. Treatment of purified CF1 with 2 M NaBr abolished its Ca2+-ATPase activity. The supernatant of 2 M NaBr-treated chloroplasts was analyzed on SDS gel electrophoresis. Figure 2 shows that it contains bands in the positions of α, β, and γ bands of CF1. These bands were so prominent that it appears that NaBr treatment removed preferentially the CF1 or its above mentioned subunits. Further identification of CF1-released subunits was carried out using their specific antibodies (14). The supernatant obtained after NaBr treatment produced on Ouchterlony plates precipi-
Fig. 1. Effect of sodium bromide treatment on photophosphorylation and Ca\textsuperscript{2+}-ATPase activities in chloroplasts. The reaction mixture for photophosphorylation contained the following in a final volume of 2 ml: 33 \textmu moles of Tricine (pH 8), 33 \textmu moles of NaCl, 13 \textmu moles of MgCl\textsubscript{2}, 6.6 \textmu moles of sodium Pi (pH 8), 2 \textmu moles of ADP, 0.06 \textmu mole of PMS, about 10\textsuperscript{5} cpn of \textsuperscript{32}Pi, and chloroplasts or NaBr-treated chloroplasts equivalent to 12 \textmu g Chl. After 1 min of illumination by white light (2.3 \times 10\textsuperscript{4} ergs per cm\textsuperscript{2} per sec) the reaction was stopped with 0.2 ml of 30\% trichloroacetic acid, centrifuged, and the supernatant assayed for incorporation of radioactivity. The reaction mixture for Ca\textsuperscript{2+}-ATPase contained the following in a final volume of 1 ml: 30 \textmu moles of Tricine (pH 8), 4 \textmu moles of [\gamma\textsuperscript{32}P]ATP, 8 \textmu moles of CaCl\textsubscript{2}, and heat-activated chloroplasts or NaBr-treated chloroplasts equivalent to 9 \textmu g Chl. After 10 min at 37 C, 0.1 ml of 30\% trichloroacetic acid was added, and after centrifugation the liberation of \textsuperscript{32}Pi was determined in the supernatant. Chloroplasts or NaBr-treated chloroplasts were heat activated at 64 C for 4 min in 0.9 ml of solution containing the following: 2.5 \textmu moles of Tricine (pH 8), 10 \textmu moles of ATP, 2.5 \textmu moles of DTT, 10 \textmu moles of sucrose, 0.3\% digitonin, and chloroplasts equivalent to 300 \textmu g of Chl.

Fig. 2. SDS gel electrophoresis pattern of purified CF\textsubscript{1} and of the supernatant after sodium bromide treatment of chloroplasts. To 2 ml of the supernatant obtained after sodium bromide treatment of chloroplasts, 0.2 ml of 30\% trichloroacetic acid was added. After centrifugation the pellet was mixed with 1 ml of acetone and centrifuged. The pellet was dissolved in 0.2 ml solution containing 10 \textmu moles tris, 2\% SDS, 2\% mercaptoethanol, and about 10\% sucrose. Purified CF\textsubscript{1} (0.6 mg) was dissolved in 1 ml of the same solution. Fifty microliter samples were applied to the gels and run for 4.5 hr at constant current of 7 mamp. per tube.
Fig. 3. Effect of sodium bromide treatment on the Hill reaction with ferricyanide. The reaction mixture contained the following in a final volume of 4 ml: 100 μmoles of Tricine-MES at the specified pH, 200 μmoles of NaCl, 10 μmoles of ferricyanide, and chloroplasts equivalent to 63 μg of Chl or 2 mM NaBr-treated chloroplasts (NaBr-P) equivalent to 88 μg of Chl. The mixture was illuminated by white light (1.5 × 10^6 ergs per cm² per sec) and the O₂ evolution was determined with a Clark type electrode. The temperature was kept at 23 C by a thermostat and a water jacket.

The electron transport of the particles was measured and high specific activities were obtained with ferricyanide (Fig. 3) or methyl viologen (Fig. 4) as electron acceptors. The pH optimum for the Hill reaction with ferricyanide was shifted to pH 6.5 and with methyl viologen to pH 7.5. These values are similar to the pH optimum for electron transport in chloroplasts in the presence of uncouplers such as NH₄Cl. NADP photoreduction was actually accelerated by the NaBr treatment (Table I). Here, too, the pH optimum was shifted to pH 7 and addition of ferredoxin NADP-reductase did not alter the rate of NADP photoreduction. The reduction was absolutely dependent upon addition of ferredoxin to the reaction mixture and plastocyanin addition had no effect.

When DCCD was included in the reaction mixture, the NaBr particles behaved exactly like untreated chloroplasts. The pH optimum for the Hill reaction was shifted back to pH 8.5, and the electron transport was accelerated by uncouplers. When light-induced pH changes were measured with NaBr particles, a light-induced proton release was observed that was reversed in the dark (Fig. 5). The amount of the released protons was about half of the light-induced proton uptake in control chloroplasts. This proton release was insensitive to uncouplers and was completely dependent on PMS. Addition of DCCD reversed the proton movement to the original light-induced proton uptake which was sensitive to uncouplers.

The pH optimum for the light-induced proton release was at pH 7.5 (Fig. 6), whereas the pH optimum for proton uptake in control chloroplasts was about pH 6 (17).

The reversal of the light-induced pH change by DCCD in the NaBr particles was time-dependent. Incubation for about 15 min at room temperature was required for 5 μM DCCD to reverse the pH effect. Increasing concentrations of DCCD shortened the lag period (Fig. 7). Table II summarizes the effect of FCCP on light-induced pH changes and on the Hill reaction catalyzed by chloroplasts and sodium bromide-treated chloroplasts. FCCP abolished the proton uptake by chloroplasts and accelerated the Hill reaction with ferricyanide and methyl viologen. In NaBr particles FCCP inhibited both Hill reaction and light-induced proton release with the former more sensitive than the latter. The light-induced proton release

Table I. Effect of Sodium Bromide Treatment on NADP Photoreduction

<table>
<thead>
<tr>
<th>Particles</th>
<th>pH</th>
<th>NADP Photoreduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>μmoles NADP/mg Chl/hr</td>
</tr>
<tr>
<td>Chloroplast</td>
<td>6</td>
<td>57</td>
</tr>
<tr>
<td>Chloroplast</td>
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<td>131</td>
</tr>
<tr>
<td>Chloroplast</td>
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<td>252</td>
</tr>
<tr>
<td>NaBr particles</td>
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<tr>
<td>NaBr particles</td>
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<td>285</td>
</tr>
</tbody>
</table>

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CHLOROPLASTS DEPLETED OF CF₁

Fig. 5. Light-induced proton release by sodium bromide-treated chloroplasts. The reaction mixture contained the following in a final volume of 4 ml: 400 μmole of NaCl, 0.5 μmole of Tricine, 0.06 μmole of PMS, and chloroplasts or NaBr particles equivalent to 30 μg Chl. The illumination and temperature control were as described in the legend of Fig. 3. The initial pH was 6.5. a: Chloroplasts; b: sodium bromide particles; c NaBr particles; and 25 μM DCCD.

Fig. 6. Effect of pH on the light-induced proton release by sodium bromide particles. Experimental conditions were as described in Fig. 5. Chloroplasts equivalent to 50 μg of Chl or NaBr particles equivalent to 30 μg of Chl were added.

by NaBr particles was sensitive to DCMU (Table III). However, the proton release cannot be explained by reduction of PMS in the light and reoxidation in the dark, because in some experiments the amount of protons released was severalfold higher than the amount of PMS added.

Fig. 7. Effect of DCCD on light-induced pH changes by sodium bromide particles. Experimental conditions were as described in Fig. 5. The initial pH was 6.5 and 2 mM NaBr particles equivalent to 30 μg of Chl were added.

Table II. Effect of FCCP on Hill Reaction and Light-induced Proton Release by Sodium Bromide Particles

<table>
<thead>
<tr>
<th>Particles</th>
<th>FCCP</th>
<th>PMS Proton Uptake or Release</th>
<th>Methyl Viologen Oxygen Uptake</th>
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<tr>
<td>Chloroplast</td>
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<td>272</td>
<td>80</td>
</tr>
<tr>
<td>Chloroplast</td>
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<td>214</td>
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<td>Chloroplast</td>
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<td>66</td>
<td>160</td>
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<tr>
<td>Chloroplast</td>
<td>7.5</td>
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<td>133</td>
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<tr>
<td>Chloroplast</td>
<td>12.5</td>
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<td>96</td>
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<tr>
<td>NaBr particles</td>
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<td>271</td>
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<td>NaBr particles</td>
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<td>-120</td>
<td>54</td>
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DISCUSSION

NaBr-treated chloroplasts lost all of their ATPase activity, which suggests that the particles are depleted of active CF₁. The SDS gels of the supernatant after the NaBr treatment showed bands in the positions of CF₂ subunits. The particles behave like uncoupled chloroplasts. The fact that this uncoupled state can be fully reversed by DCCD suggests that
suggested that specific binding sites for protons play a role in the over-all light-induced proton movements in chloroplasts (4, 5, 7, 9). If the NaBr particles were permeable to protons and probably to other monovalent ions the light-induced proton release might be caused by sodium and H⁺ exchange on specific sites within the membrane. In the light, Na will exchange bound protons which will result in pH decrease, while in the dark the reverse of this reaction takes place and the pH is rising again. Alternatively, light-induced conformational changes in the membrane may expose bound protons and release them to the aqueous medium. In the dark the conformation is reversed and the protonated functional groups are buried in the lipid phase of the membrane. This might also explain the light-induced changes in buffer capacity of chloroplasts observed by Polya and Jagendorf (18).

The NaBr particles might prove useful as a tool not only for the study of the energy transfer machinery but also to get better understanding of the interaction of protons with functional groups within the membrane.

Acknowledgments—We wish to thank Mr. F. J. Mallett for his support of this project. The technical assistance of Mrs. Bat-El Notani is gratefully acknowledged.

LITERATURE CITED


